Rational Roots of Sympathetic Overactivity by Neurogenic Pulmonary Edema Modeling Arising from Sympathyco-Vagal Imbalance in Subarachnoid Hemorrhage: An Experimental Study

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■ BACKGROUND: **Autonomous innervations of the lungs are maintained by cervical sympathetic and vagal nerves. Sympathetic overactivity-induced neurogenic pulmonary edema (NPE) is known as a serious complication of subarachnoid hemorrhage, but the rational neuronal mechanism of that overactivity has not yet been clarified fully. The aim of this study was to examine whether there is a relationship between vagal nerve ischemia related sympathetic overactivity and neurogenic pulmonary edema in subarachnoid hemorrhage.**

■ METHODS: **This study was conducted on 27 rabbits. A control group was formed of 5 animals, a sham group of 7 to which saline was administered, and a study group of 15 animals that were injected with homologous arterial blood into the cisterna magna. Electrocardiography and respiratory rhythm parameters were monitored for 3 weeks and the animals were then decapitated. Statistical analysis was made of the numbers of degenerated axons in the pulmonary branches of the vagal nerves, the neuron density of stellate ganglions and the vasospasm index of the pulmonary arteries.**

■ RESULTS: **In the control group, the normal respiration rate was 34 ± 6 bpm, total axon number was 1600 ± 270/mm^2, degenerated axon number was 10 ± 3/mm^2, and vasospasm index was 1.34 ± 0.25. The sham group values were 30 ± 3 bpm, 163 ± 47/mm^2, and 1.95 ± 0.45 and the study group values were 45 ± 8 bpm, 530 ± 92/mm^2, and 2.76 ± 0.83. The mean stellate ganglion neuron density was evaluated as 8.112 ± 1.230/mm^3 in all animals, as 7.420 ± 4.10/mm^3 in animals with slight NPE, and as 12.512 ± 1.236/mm^3 in animals that developed severe NPE.**

■ CONCLUSION: **High neuron density of stellate ganglion may have important roots in sympathetic overactivity-related NPE development in subarachnoid hemorrhage.**

INTRODUCTION

Respiration can be defined as the transport of oxygen and carbon dioxide between cells and the external environment through inspiration and expiration.[1,2] The innervation of respiratory organs is supplied by both the somatic and autonomic nervous systems.[3] The conscious continuation of respiration is regulated by the somatic sensitive motor nerves. These nerves originate from the lower brainstem and the cervical and thoracic segments and control the respiration muscles and pleural and thoracic surfaces.[2] Autonomic nerves regulate the diameter of the conducting airways, pulmonary blood vessels, the volume of respiratory units, and the bronchial glands and reflexes. The major neural pathway between the brainstem and lungs is innervated by the vagal nerves. They play a major role in regulating the rhythm of respiration and the regulation of pulmonary vessels, airways, and pulmonary secretion.[4] Vagal stimulation or activation of the pulmonary stretch receptors by lung inflation triggers the Hering-Breuer reflex. This reflex inhibits inspiration and lengthens the period of expiration.[5] Activation of the vagal nerve results in

Key words
- Respiration
- Subarachnoid hemorrhage
- Vagal ischemia

Abbreviations and Acronyms
H&E: Hematoxylin and eosin
NPE: Neurogenic pulmonary edema
SAH: Subarachnoid hemorrhage
VSI: Vasospasm index

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bronchoconstriction, respiration rhythm disorders, and apneustic attacks.[6] When respiration units are irritated by chemical, pathophysiologic, or mechanical factors, the respiration reflex is triggered, which alters the pattern of breathing.[7,8] According to these data, at the beginning of subarachnoid hemorrhage (SAH), there can be ischemic vagal changes, which act as vagal activation and trigger the apneustic attack. Vagal nerve injury disrupts vagal nerve regulation, which disturbs the respiratory rhythm.[9]

Vagal nerve damage causes paralysis of the laryngopharyngeal muscle and disruption of tracheobronchial structures, resulting in reflex vagal bradycardia and bradypnea.[10] In late-phase SAH, irreversible axonal injury occurs and resembles a blockade of the vagal nerves. Acutely developed cerebral ischemia causes degeneration through the visceromotor pulmonary branches of the vagus. Restoration and some healing has been observed in some animals.[11,12] The volume of the vagal nerve is crucial for its activity.[13] The volume of autonomic ganglions also affects the autonomic functions.[13] According to these data, the volume of the vagal nerve is crucial for respiration. In SAH, there is a reduction of the volume of the vagal nerve, which can cause respiration problems and even respiratory arrest.

In the current study, it is demonstrated that SAH causes not only cerebral ischemic insult but also vagal ischemia. The hypothesis of our study was that sympathetic overactivity based on ischemic vagal network injury may originate from high neuron density of the stellate ganglion, and therefore high neuron density is an important factor in sympathetic overdischarges and the development of respiratory arrhythmia and neurogenic pulmonary edema (NPE). To the best of our knowledge, high neuron density is a novel concept that has not been studied previously.

MATERIALS AND METHODS

This study was conducted at the Medical Experimental Research Center, Ataturk University. Approval of the study protocol was granted by the Ethics Committee of Ataturk University. All procedures were performed in accordance with the National Institute of Health Principles of Laboratory Animal Care. A total of 27 hybrid rabbits, each aged 2 years and weighing 3.5 ± 025 kg, were used. A control group was formed of 5 animals, a sham group of 7 to which saline was administered, and a study group of 15 animals, which were injected with homologous arterial blood into the cisterna magna.

All animals were fasted for 6 hours before surgery. A balanced injectable anesthesia was used to reduce pain and mortality. After we induced anesthesia with isoflurane administered via a facemask, 0.2 mL/kg of the anesthetic combination (ketamine HCL, 150 mg/1.5 mL; xylazine HCL, 30 mg/1.5 mL; and distilled water, 1 mL) was injected subcutaneously immediately before surgery. All the animals were monitored for electrocardiographic changes, respiration patterns, and blood oxygen concentrations during the procedures. All parameters were recorded by a Sony Camera (Model SLT-A65V, Tokyo Japan) and were analyzed by physicians who were blinded to the study.

During the operation, 0.1 mL/kg of anesthetic combination was used as required. For the SAH group, 0.75 mL of autologous blood was taken from the auricular artery and injected into the cisterna magna via a 22-gauge hypodermic needle over approximately 1 minute. For the sham group, 0.75 mL of isotonic saline solution was injected instead of blood. Of the 15 rabbits subjected to experimental SAH, 2 died within the first week after surgery and the surviving 13 were followed for 3 weeks without any medical treatment and then sacrificed. At that point, the lungs and all vagal nerves just over the lung hylus were removed for histological examination. Tissues were kept in a 10% formalin solution for 7 days, following which 1 μm tissue sections were taken and stained with hematoxylin and eosin and S-100. Tissue specimens were stained with hematoxylin and eosin to detect the localization of pulmonary vagal branches in the hylar region. The S-100 technique was then used to differentiate normal and degenerated axon numbers of the pulmonary branches of the vagal nerves. Thick, brown axons were accepted as normal and the others were accepted as degenerated.

To estimate the total and degenerated axon numbers of vagal nerves, the fractionation technique was used after l cross section of the vagal nerves was obtained according to the basic sampling procedure and the stereological principles described by Gundersen et al.[14] Axon density of the vagal nerve was estimated by the use of an unbiased counting frame of known size. The microscope used had 2 attachments; a camera and a platform with 2 dial indicators and 2 arms for mounting the microscope stage. The 2 dial indicators (5-μm resolution) mounted to the microscope measured movement in the x and y lines separated by 45° of the stage. The vagal nerve images and measurements obtained were recorded by the camera and uploaded to a computer, and the numbers of normal and degenerated axons of the vagal nerves stained with S-100 were counted.

The following pathologic and histologic findings are accepted as NPE criteria. In macroscopic samples, intraparenchymal lung hemorrhages and foamy hemorrhagic effusions were observed in all animals. Loss of ciliary and alveolar cells, intra-alveolar hemorrhage, alveolar wall ruptures, vascular congestion, and vasoconstricted artery branches were seen in the histopathologic samples of the lungs of all animals with NPE.

To estimate respiration capacity and depth, respiration—time curves generated by computer were overlaid onto paper marked out with mini-squares to facilitate the calculations. The height of the curve from the basal line to the apical points was accepted as respiration depth, and the surface area under the curve was accepted as respiration capacity per respiration cycle recorded during a period of 2 seconds. Then, these values were multiplied by 30 to calculate the mean value per minute. The numbers of degenerated vagal nerve root axons, respiration depths, and respiration capacity values were compared between the 3 groups with the Mann-Whitney U test.

RESULTS

Two of the SAH-induced animals and 1 of the sham group animals died within the first 7 days postoperatively as the result of respiration arrest, and the remaining animals (SAH, $n = 13$; sham, $n = 6$) were followed up for a total of 21 days postoperatively. Clinically, signs of meningeal irritation, unconsciousness, convulsive attacks, fever, apnea, cardiac arrhythmia, and breath disturbances were observed frequently in the premortal periods of all the animals who died within the first week and in 3 of the animals who survived for the full 21 days. Figure 1 shows the normal anatomical